THE ROLE OF THE SINUS GLANDS IN RETINAL PIGMENT MIGRATION IN GRAPSOID CRABS

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Introduction

Among the several functions ascribed to a hormone or hormones produced by the sinus gland of the crustacean eyestalk is that of controlling the migration of the retinal pigments. Evidence for this function has been presented chiefly by Bennitt (1932a, b), Kleinholz (1934, 1936), and Welsh (1939, 1941) and is well summarized in the reviews of Kleinholz (1942), Brown (1944), and Panouse (1947). Briefly stated, the compound eyes of decapod crustaceans typically possess three sets of pigment cells: distal melanophores forming a sleeve about each ommatidium, proximal melanin contained within the retinular cells (photoreceptive cells, proximal pigment cells), and reflecting pigment cells located among and beneath the retinular cells. In day-adaptation the proximal and distal melanins approach each other so as to surround the sensitive rhabdomes, while the reflecting pigment may be shifted proximally beneath the basement membrane of the retina. In night adaptation the proximal and distal melanins move apart in such a way as to leave the rhabdomes exposed to light from all sides, while the reflecting pigment is exposed to form a reflecting layer at the bases of the rhabdomes, visible as a reddish area of "glow" in the dark-adapted eye. The relative extent of movement of the three types of pigment differs in detail in various species of crustaceans. Attempts to demonstrate nervous control of retinal pigments in crustaceans have been unsuccessful, while the possibility that these cells are independent effectors is not supported by the available evidence. In particular, the marked diurnal movements of retinal pigments exhibited in the eyes of many crustaceans under conditions of constant darkness or of constant illumination would seem to rule out independent response to illumination as anything but an incidental factor in the normal process of pigment migration. Positive evidence for a hormonal control is based upon the discovery by Kleinholz (1934, 1936) that extracts of light-adapted *Palaemonetes* eyestalks injected into dark-adapted animals in darkness caused the distal and the reflecting pigments to move to the light-adapted position. This work was confirmed on *Cambarus* by Welsh (1939), who found that the proximal pigment was likewise induced to migrate to the light-adapted position if sufficiently strong injections of eyestalk extract were used. It has generally been assumed that the sinus gland is the source of the retinal pigment activator, and Welsh (1941) has shown that aqueous extracts of isolated sinus glands are capable of causing typical pigment migration when injected into dark-adapted crayfish. As will be reported below, similar results may be obtained in grapsoid crabs.

While the evidence for a hormonal control of crustacean retinal pigments is generally convincing, this concept has been based upon procedures which have not

included one of the classical tests of endocrine function, namely, the removal of a suspected organ to produce characteristic symptoms, followed by the injection of extracts or the implantation of the organ to restore the normal conditions. Furthermore, most experimental work has been carried out with macrurans. Accordingly, it was felt that a further study of brachyurans would be of value, and that sinus gland extirpation should be made the main line of approach to the problem.

If the sinus gland is the source of retinal pigment activating hormone, its removal would be expected to stop diurnal pigment migrations and to maintain the eye in a dark-adapted state. Continued display of retinal pigment migration after sinus gland removal would, on the other hand, indicate that this organ is not the controlling factor or at least not an indispensable link in the process. Welsh (1941) has given evidence that the control of diurnal changes in retinal pigments involves central nervous activity, possibly affecting the release of hormone by the sinus gland, but the possibility that this effect can be mediated in the absence of the glands has not been investigated. The present paper reports attempts to clarify the role of the sinus glands in retinal pigment migration by means of operative sinus gland removal.

Material and Methods

A. *Animals*

Three species of grapsoid crabs common in the San Francisco Bay area have been employed: *Hemigrapsus oregonensis, H. nudus,* and *Pachygrapsus crassipes.* These animals are hardy, possess well-defined sinus glands, and show a marked "glow" in the eyes in darkness at night. With the exception of the work of Bowman (M.A. Thesis, U. C., Berkeley, Calif., 1948) no previous study of the pigmentary changes and endocrine functions of this group of crabs has been made. In order to reduce possible complications associated with egg-bearing, chiefly males have been used in the work, although enough females have been observed to indicate that their pigmentary responses are the same as displayed by the males. Animals were selected for size, since those less than 17 mm. in carapace width were too small for convenient sinus gland removal, while those exceeding 30 mm. in width were not only too large and active for easy handling under the dissecting microscope, but also possessed such thick and pigmented exoskeletons as to render difficult the observations on body chromatophores sometimes carried on concurrently with studies of retinal pigments.

All animals were kept in shallow water in individual covered glass dishes, were fed fresh liver, clam, or crab meat and had the water changed every 3 or 4 days.

B. *The preparation of active extracts*

Sinus glands and other organs to be tested for retinal pigment activating effect were taken from animals of approximately the same size as the recipients, so that dosage could be estimated in terms of a fraction of the organ in question. Recipient crabs were measured or weighed before an experiment, and a group of donor animals selected so as to have a slightly greater average width or weight than the recipients. Eyestalks were removed, split open in crab perfusion fluid, and the chain of optic ganglia with the attached sinus gland teased free with fine forceps. The sinus gland was detached and removed to a drop of distilled water in a covered one-inch Syracuse dish. The medulla terminalis was similarly placed in a second dish, and the

group of three more distal ganglia (medulla interna, externa, and lamina ganglionaris) in a third dish. Other tissues extracted included brain, which in these crabs has about twice the mass of the medulla terminalis, thoracic ganglionic mass, and claw muscle. The respective tissues, when a sufficient amount had been collected, were ground with rounded glass rods, although the small sinus glands ordinarily escaped thorough crushing. Crab perfusion fluid was added to the dishes with a 1 cc. hypodermic syringe, stirred, and then taken back into a marked syringe for transfer to a small test tube. The volume of fluid used being known, a further quantity of perfusion fluid sufficient to dilute the suspension to the desired degree was added to the Syracuse dish, and likewise transferred with the marked syringe to the test tube. Ordinarily, 10 or 20 sinus glands, medullae terminalis, and distal ganglia were used, and made up to give extracts containing the equivalent of 10 organs in one cc. Brains were diluted to 5 per cc., while thoracic nerve mass and claw muscle were dissected out in quantity judged to be a little greater than the mass of brain tissue used. After transfer to test tubes, all extracts were heated in boiling water for 10 minutes, cooled, and used unfiltered. Injections were made at the base of a walking leg, the quantity injected being 0.05 cc., using for each extract the same syringe used in handling that material in preparation. Extracts stored in a refrigerator retained their potency for several days.

C. *Determination of the relative size of extracted organs*

In order to arrive at a dilution factor, so that extracts could be diluted to contain a bulk of tissue comparable to that present in an extract of sinus glands, there were dissected out in turn from each eyestalk of four crabs the sinus gland, the medulla terminalis, and the group of three distal optic ganglia. These organs from each eye were placed on a haemocytometer slide in crab perfusion fluid and compressed under the cover glass (0.1 mm. clearance). Camera lucida tracings on good quality graph paper were made, the outlined areas being cut out and weighed in groups. A test made by cutting out squares of known area showed that the combined errors in cutting and in thickness of the paper did not exceed 1.5 per cent, whereas variations in dissection were large. In Table I the relative weights of the separately dissected organs are tabulated, showing the wide variation that may be expected in removing

TABLE I

Relative sizes of sinus gland and optic ganglia

Animals		Sinus gland		Med. terminalis		3 distal ganglia	
Width	Sex	Right	Left	Right	Left	Right	Left
16.5 mm.	♀	93	97.5	1020	1070	1120	1610
17 mm.	♀	109	51	1320	1290	1690	1630
17 mm.	♂	34	26.5	1790	1300	1980	1700
19 mm.	♂	75	79	1650	1480	1650	lost
Total weights		565		10,920		$11,380 \times 8/7 = 13,006$	
Ratio		1		19.3		23	

these small, soft organs, and the necessity of using sufficient numbers of organs in an extract to ensure even approximate consistency in results.

There are two sources of error which contribute to making the relative size of the sinus gland appear larger than is actually the case:

(1) While the ganglia could be picked free of adherent tissue, some extra tissue generally was included with the tiny sinus glands.
(2) The small size of the sinus gland allowed it to rest in the 0.1 mm. space between haemocytometer slide and cover glass without appreciable flattening. The projection of its outline thus encompassed some tissue actually less than 0.1 mm. in thickness.

These sources of error may make the sinus gland appear perhaps nearly twice its true size, relative to the optic ganglia. For purposes of dilution, however, it was assumed to have a bulk of $\frac{1}{20}$ that of the medulla terminalis and of the group of distal ganglia.

D. *Estimation of degree of dark-adaptation*

This was done by noting the extent of "glow," or light-reflecting area in the eye. Animals to be examined were picked up in dim red light and their eyes examined with the aid of a 6-volt "Mignon" lamp held close to the observer's line of vision, an operation requiring only a few seconds. Because of the irregular shape of the crab "cornea," it is difficult to make a quantitative estimate of the amount of "glow" present, especially since the "glow" is more pronounced and more often seen in the most ventral portion of the retina than elsewhere. The following scheme of expressing the degree of dark-adaptation in retinal pigments was followed, with the realization that the four stages and one doubtful intermediate stage (±) indicated are not necessarily proportional to the activity of the photo-adaptive mechanism:

Symbol	Description
0	Fully light-adapted. No glow visible.
±	Indistinct glow in ventral region of one or both eyes.
+	Glow distinct, but restricted to ventral region of eye.
++	Glow distinct in ventral region, with sub-maximal glow visible in long axis of eyestalk.
+++	Fully dark-adapted. Pronounced glow visible in long axis of eyestalk.

The use of "+" symbols was convenient in recording retinal pigment positions in those cases in which the state of body chromatophores (indicated by numbers 1–5) was studied in the same animals. The diurnal pattern of retinal pigment migrations was plotted with respect to time, the resulting curve serving to show the general trend of events. Since only three or four observations were made per day in order to avoid excessive stimulation by handling and bright light, the curves (whose exact form is of little importance in the present study) are a somewhat stylized expression of the day-to-day pattern of retinal pigment migration.

E. *Sinus gland extirpation and control operations*

Experimental animals were operated upon by means of a small dental excavating burr mounted in a jeweler's screwdriver handle, permitting the drill to be rotated

with the fingers of one hand. With the crab securely wrapped in wet cloth and held under the medium power of a dissecting microscope, a hole somewhat less than 1 mm. in diameter was drilled through the dorsal side of the eyestalk. The pigmented hypodermis was then slit and laid aside with a needle, revealing (if the hole were correctly located) the sinus gland, easily recognized by its glistening bluish-white appearance against the gray of the optic ganglia. The upwelling of blood usually raised the gland so that it could be teased free with a needle or sharp-pointed watchmaker's forceps and removed. In our experience the removal of sinus glands from these crabs is easier than from the crayfish, because of the superficial location and more compact form of the gland. The wound was cauterized superficially to coagulate the blood across the opening to reduce bleeding. It was found best to wait several hours after the first operation before removing the second sinus gland. In about 10 per cent of cases the gland was broken in removal, necessitating considerable probing to retrieve all portions of it, and causing much bleeding. In cases where the opening was cut at a point not over the gland, it proved best not to attempt to seek out the gland, but to use the operation as a control. In control operations the ganglia proximal to the sinus gland were in some cases slashed with a needle point in the effort to sever the nerve supplying the gland without cutting the optic tracts; in other cases the entire optic tract was severed. The extent of injury inflicted in sinus gland removal on small crabs proved to be difficult to evaluate but was less than we have experienced in carrying out sinus gland removal in crayfish following the method of Brown (1942). The sinus gland could generally be picked from its superficial position with no apparent damage to nerves and good healing took place, yet serial sections generally revealed some degree of nerve injury, often severe enough to make it impossible to attribute the observed results simply to the absence of the sinus gland. Eyestalks were routinely fixed and sectioned serially after observations, but because of the number and the refractoriness of the heavy-walled crab eyestalks, the results of sectioning left much to be desired.

Mortality from operating was not heavy, except in the case of animals still soft from a recent moult, or those which moulted within a few days after an operation. Among animals not so weakened, deaths from bilateral sinus gland removal have amounted to 11 per cent and from operations involving nerve sectioning to 19 per cent, in some 35 and 25 animals respectively, operated upon in the series of experiments reported here. The writer recognizes that the method of sinus gland removal employed upon these small crabs does not permit the control of bleeding and minimization of nerve injury attainable with the more precise methods which Kleinholz (1947) has used upon crayfish and lobsters. It was felt, however, that results obtained on large numbers of small, responsive, easily kept animals would aid in the definition of problems to be attacked later in larger crabs kept under better conditions than have been available locally.

Observations

A. *The normal rhythm of retinal pigment migration*

Normal crabs kept in the laboratory showed the characteristic changes in the position of the retinal pigments in response to the daily alternation of light and darkness, exhibiting a well-marked glowing "pseudopupil" in the eyes at night and the absence of such during daylight hours. This migration stopped under continuous

illumination (tested in a windowless room illuminated by two 150-watt bulbs in globes 7 feet above the animals), and the crabs remained in a continuously light-adapted state, not showing the diurnal rhythm of retinal pigment activity reported for certain other crustaceans. On the other hand, crabs kept in continuous darkness exhibited the typical diurnal rhythm of retinal pigment migration, becoming fully dark-adapted during the night hours and fully light-adapted during the day.

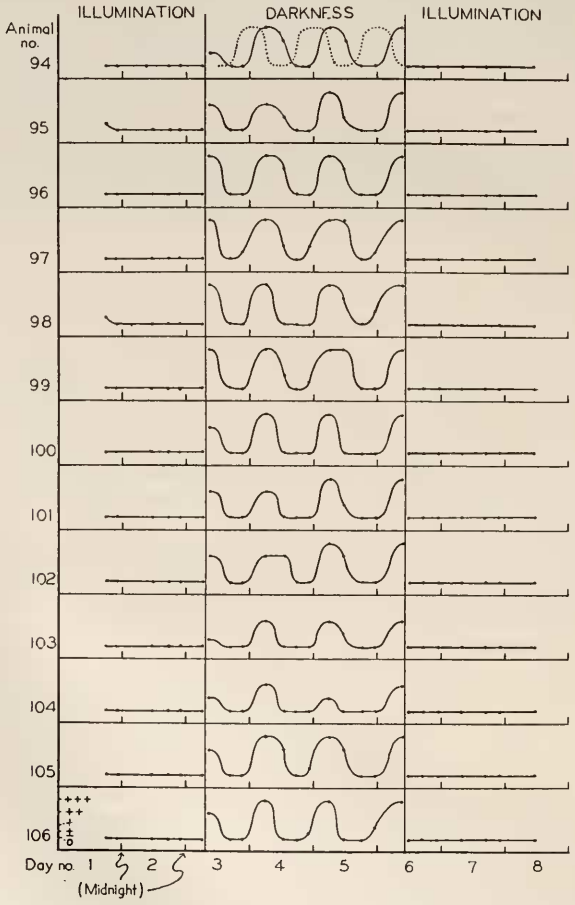

FIGURE 1. Diurnal migrations of retinal pigments exhibited in darkness but absent under illumination. Rhythm in darkness put out of phase with solar day by starting dark-adaptation at dawn. Dotted curve represents a "normal" rhythm.

This activity continued undiminished for at least 10 days, although occasional animals gradually became "out of phase" with the solar day. Ordinarily night-adaptation commenced in the late afternoon, even before sunset, while the reverse process of day-adaptation was often under way before dawn. The most striking case of a shift in phase of the diurnal activity cycle occurred when a group of *Hemigrapsus oregonensis* which had been exposed to continuous light for 40 hours were

transferred to the darkroom at 7 A.M. They soon became dark-adapted, and thereafter exhibited a period of dark-adaptation starting after midnight and lasting until early afternoon—an example of the reality of a diurnal activity rhythm as well as of its lability (Fig. 1).

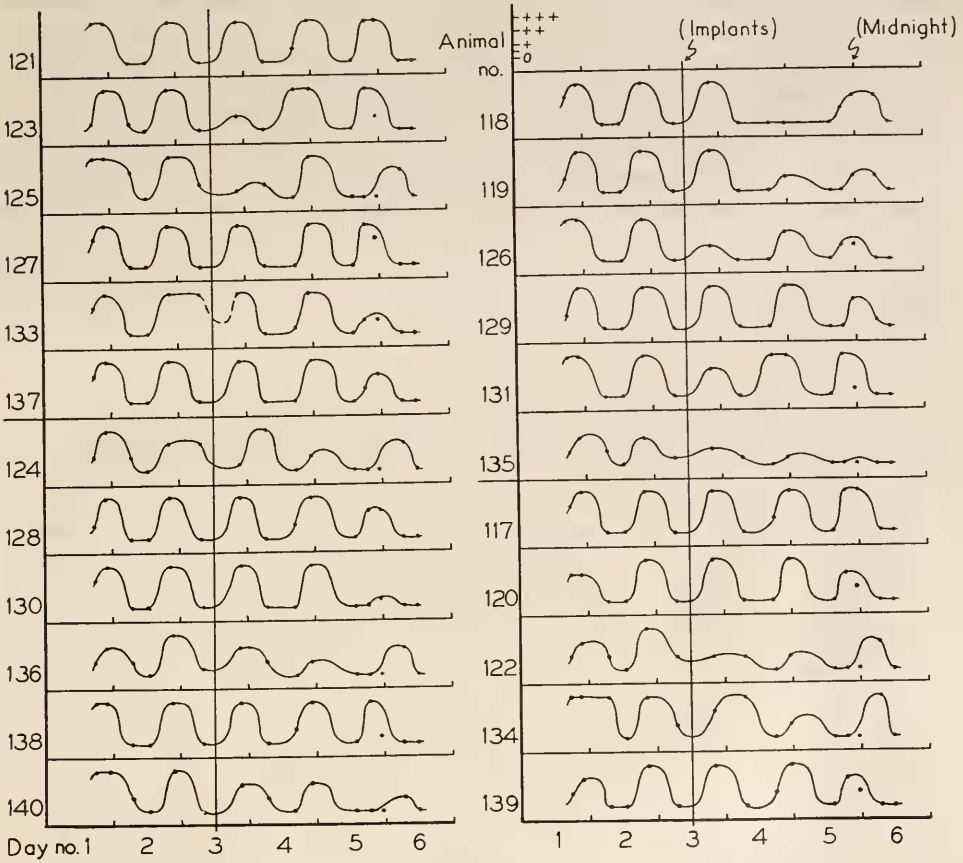

FIGURE 2. Relative ineffectiveness of a sinus gland or other tissue (implanted on third day) in suppressing diurnal rhythm of retinal pigments of crabs kept in darkness. 121–137, one sinus gland; 124–140, one medulla terminalis; 118–135, one group of three distal optic ganglia; 117–139, muscle. Note that on the fifth night too close spacing (95 minutes) between observations decreased the degree of dark-adaptation obtained.

B. *The effects of injections of sinus gland and nervous tissue extracts*

Over 40 tests were made in which sinus glands or optic ganglia were implanted by the technique of Scudamore (1947, p. 190) into the bases of walking legs of crabs the same size as the donors. The recipient crabs were observed in constant darkness to determine if the implanted tissues would suppress the normal diurnal cycle of the retinal pigments. While there was some evidence that a factor active upon retinal pigments was present in nervous tissues as well as in sinus glands, the results were not conclusive, as shown in Figure 2, which is typical of the whole series. On

the other hand, extracts of whole eyestalks injected into night-adapted animals of all three species in darkness caused a migration of retinal pigments to the "day" position, as evidenced by a complete or nearly complete disappearance of the "glow." Since it had early been observed that if crabs were inspected for "glow" in the darkroom at night, there often occurred a transitory change to a partially light-adapted state within the hour or two after the illumination of the eyes (as shown in Fig. 2 fifth night), and since these crabs only became dark-adapted during the night hours it was necessary to time injections in such a way that all animals could be safely assumed to be night-adapted at the time of injection without making an inspection. The procedure adopted was as follows: a group of 24 crabs were measured, placed in numbered dishes, and brought into the darkroom at about 6 P.M. Inspection at a later hour that evening and again during the following day sufficed to show that the animals were exhibiting the normal diurnal rhythm. They were then presumed to be night-adapted by 9 or 10 P.M. of the second evening. Injections were made under dim red light, the time of each being noted. At one hour after injection, each animal was inspected for "glow." This procedure was repeated the next two eve-

TABLE II

Effects of sinus gland and optic ganglia extracts, tested upon Hemigrapsus oregonensis

Crab no.	Width (mm.)	March 7		March 8		March 9	
		Injection	Result	Injection	Result	Injection	Result
1	21	Control injection	+++	½ sinus gland	+	Muscle	+++
2	19		++		0		++
3	17		+++		±		+++
4	19		+++		+		+++
5	20	½ medulla terminalis	+	Control injection	+++	½ sinus gland	Died
6	18		+		+++		0
7	17		+		+++		+
8	20		+		+++		±
9	19	1/40 medulla terminalis	++	½ medulla terminalis	+	Control injection	+++
10	17		++		+		+++
11	21		++		+		+++
12	18		++		+		+
13	20	½ distal ganglia	+	1/40 distal ganglia	+++	½ medulla terminalis	±
14	19		+		++		±
15	16		±		+++		±
16	18		+		+++		+
17	21	Muscle	+++	½ distal ganglia	+	1/40 sinus gland	+
18	18		+++		+		++
19	17		+++		±		+
20	18		+++		+		++
21	17	½ sinus gland	+	Muscle	+++	½ distal ganglia	0
22	18		+		+++		+
23	17		±		++		±
24	17		0		+++		0

nings, so that injections were given to the same group of crabs for three successive nights. Since the injections often interrupted the normal pattern of diurnal migration of pigments, it was surprising that consistent results could be obtained even for three nights. After such a series of injections, the test animals were discarded, since the tendency to get out of phase with the solar day introduced an element of uncertainty.

There appear, in Tables II and III, the results of two such series of injections of extracts of sinus gland, nervous, and muscular tissues. Extracts were from light-adapted crabs of the same species and of the same average size as the recipients, and all injections were of 0.05 cc. of extract, the concentrations being expressed as the fraction of an "average" organ injected. A given extract was used for one series of three successive injections. Control injections were of crab perfusion fluid.

TABLE III

Effects of extracts of brain, thoracic nerve mass, optic ganglia and sinus gland, tested upon Hemigrapsus oregonensis

Crab no.	Width (mm.)	March 14		March 15		March 16	
		Injection	Result	Injection	Result	Injection	Result
25	18	Brain ½ hemisphere	+	Brain 1/40 hemisphere	++	1/10 thoracic nerve mass	+
26	20		++		+++		±
27	19		+		+		Died
28	21		+		++		+
29	17	1/10 thoracic nerve mass	++	1/200 thoracic nerve mass	++	½ medulla terminalis	±
30	18		++		+++		±
31	17		++		++		±
32	20		++		++		+
33	18	½ medulla terminalis	±	1/40 medulla terminalis	++	Muscle	+++
34	18		±		++		++
35	18		+		++		+++
36	19		++		++		+++
37	18	Muscle	+++	Muscle	+++	½ sinus gland	0
38	18		+++		+++		±
39	18		+++		+++		0
40	21		+++		+++		±
41	17	½ sinus gland	+	1/40 sinus gland	+	Control injection	+++
42	21		+		+		+++
43	16		±		+		+++
44	17		±		++		++
45	17	Control injection	+++	Control injection	+++	Brain ½ hemisphere	+
46	20		+++		++		±
47	17		+++		+++		Died
48	20		+++		+++		+

While these injection methods are only roughly quantitative, several generalizations may be drawn from the results:

(1) The total retinal pigment activating potency of one sinus gland is roughly equivalent to that found in one medulla terminalis, or in one group of the three more distal optic ganglia.

(2) Since the bulk of either of these ganglionic masses is at least 20 times that of the sinus gland, it would appear that the active principle is at least 20 times more concentrated in the latter organ.

(3) Of the total active substance extractable from the eyestalk, roughly ⅓ resides in the sinus gland. This is in contrast to the distribution of body-chromatophore-activator in the eyestalks of several species of shrimps and crabs studied by Brown (1940) in which at least 80 per cent of the total activity resides in the sinus gland.

(4) Nervous tissue other than optic ganglia also contains a retinal pigment activating principle, in a concentration of the same order of magnitude as is found in optic ganglia.

The above facts suggest that the production of retinal pigment activator is not restricted to the sinus gland, but occurs in other parts, or perhaps all parts, of the nervous system. The active substance may, however, be more efficiently produced or quantitatively stored in the sinus gland, which in turn might be specialized for its periodic release. Upon this basis, attempts were made to block the diurnal retinal pigment changes by operative removal of sinus glands.

C. *Sinus gland removal in animals exposed to normal darkness and daylight*

Bilateral sinus gland removal was carried out upon a group of 13 crabs and the animals observed daily and nightly for periods of 3–5 days. The presence or absence of "glow" was noted but sufficient data to show the time course of pigment migration were not collected. The results of sinus gland removal, as observed in the living animals, were quite variable, but in no case did a glow appear during daylight as the result of the operation. A glow was generally present at night, but was variable in amount, ranging from a practically normal glow down to a slight or questionable glow. Both eyes of each of these animals were fixed and sectioned serially, one eye being removed in bright daylight, and the other at night, under dim red light. A summary of the observations with the results of a study of serial sections of the eyestalks follows (Table IV).

As a control operation, the optic tract was severed within the eyestalks of 8 animals by the severe procedure of stabbing the region of the medulla terminalis or interna with a heated needle, after drilling a hole as for sinus gland removal. The sinus gland itself was not touched. With two exceptions the animals in this group failed to show night adaptation. Histological examination revealed that in at least three animals, possibly because of damage to blood supply, the retina and distal optic ganglia were in a necrotic state. In such animals the apparent state of light-adaptation is perhaps comparable to that reported by Bennitt (1924) as due to death or to separation of the eyestalk from the body rather than to processes related to denervation or interference with sinus gland function. The results obtained on the remaining five animals are summarized in Table V.

The effect of optic nerve severance seems clearly to be a continued state of day-adaptation, a result consistent with the view advanced by Welsh (1941) that the light-adapted state is brought about by the release of a sinus gland hormone when a tonic nervous inhibition of the gland is reduced. Yet the view that a sinus gland

TABLE IV

Results of histological examination; sinus gland removal; normal daylight and darkness

Record number	Species	Response	Sinus glands	Nerve damage
9	*H. oregonensis*	Normal	Complete removal	Slight
11	*H. oregonensis*	Right: normal Left: impaired night adaptation	Complete removal	Right: slight Left: considerable
13	*H. oregonensis*	Impaired night condition	Complete removal	Right: optic tract severed Left: some damage
16	*H. oregonensis*	Right: intermediate Left: normal	Complete removal	Right: necrotic Left: slight damage
18	*P. crassipes*	Normal	Complete removal	Slight
21	*P. crassipes*	Impaired night adaptation	Right: removed Left: some remaining	Right: slight Left: severe
24	*H. nudus*	Normal	Complete removal	Slight
39	*H. nudus*	Impaired night adaptation	Complete removal	Right: severe Left: considerable
40	*H. nudus*	Normal	Complete removal	Slight
46	*H. nudus*	Impaired night adaptation	Complete removal	Moderate to severe
47	*H. nudus*	Right: normal Left: impaired night adaptation	Right: some remaining Left: removed	Right: slight Left: optic tract severed (?)
49	*H. nudus*	Impaired night adaptation	Right: trace remaining Left: removed	Slight
50	*H. nudus*	Impaired night adaptation	Right: trace remaining Left: removed	Slight

hormone is solely responsible for inducing the movement of pigment to the "day" position is not supported by the results of sinus gland removal, which in no case caused the eye to become permanently "dark-adapted;" in fact any changes that were produced were in the direction of increased day-adaptation, a result possibly attributable to nerve damage incidental to sinus gland removal. Furthermore, the possibility exists that, in the absence of sinus gland hormone, the retinal pigments might possess some power to act as independent effectors in a direct response to light. Accordingly other series of observations were made under conditions of continuous darkness.

TABLE V

Results of histological examination; optic nerve sectioning; normal daylight and darkness

Record number	Species	Response	Sinus glands	Nerve damage
12	*H. oregonensis*	Permanent "day" adaptation	Damaged (?)	Optic tracts severed
25	*P. crassipes*	Permanent "day" adaptation	Intact	Optic tracts severed
41	*H. nudus*	Permanent "day" adaptation	Right: intact Left: damaged	Right: optic tract severely damaged Left: optic tract severed
42	*H. nudus*	Right: day adapted Left: nearly normal	Intact	Right: optic tract severed Left: optic tract severely damaged
45	*H. nudus*	Right: day adapted Left: impaired night adaptation	Intact	Right: optic tract severed Left: optic tract damaged

D. *Sinus gland removal in animals exposed to continuous darkness*

The effects of sinus gland removal and optic tract damage were tested upon a group of 25 animals of all three species, both males and females, selected to include no soft, recently moulted crabs. In the course of the experiment, five moulted and did not survive. Observations were first made on the intact animals for three days and nights, to obtain a record of the pattern of the diurnal rhythm in each animal (see Fig. 3). On the fourth day the group was returned to a lighted room, fed, given fresh seawater, and received the first operation upon the right eyestalk. On the following day the second operation was performed on the left eyestalk, and the animals returned to darkness. Observations continued over the next five days and nights (with one night on which no check could be made). By reference to Figure 3 it may be seen that in the four normal control animals the rhythm continued unchanged.

The results of operations again showed considerable variation. In this series control operations were restricted to a superficial cut across the optic tracts proximal to the sinus glands, in the effort to denervate the gland without severing the main optic tracts. Of the six survivors, four showed no real change, while in two animals (nos. 57 and 63) one eye continued to show diurnal changes and the other did not. Histological examination of the eyes in this group seemed to indicate that in those two eyes which failed to show pigment migration, nerve damage was more severe than in those eyes which continued to show diurnal pigment migration, but the evidence was far from satisfactory. The fact that the two eyes of a single animal may show different degrees of adaptation has been noted often enough in this work to indicate a considerable degree of independent control of the pigments in each eye.

Ten animals survived bilateral sinus gland extirpation (of these, nos. 54, 65, and 76 had the left eyestalk removed, observations being carried out on the right

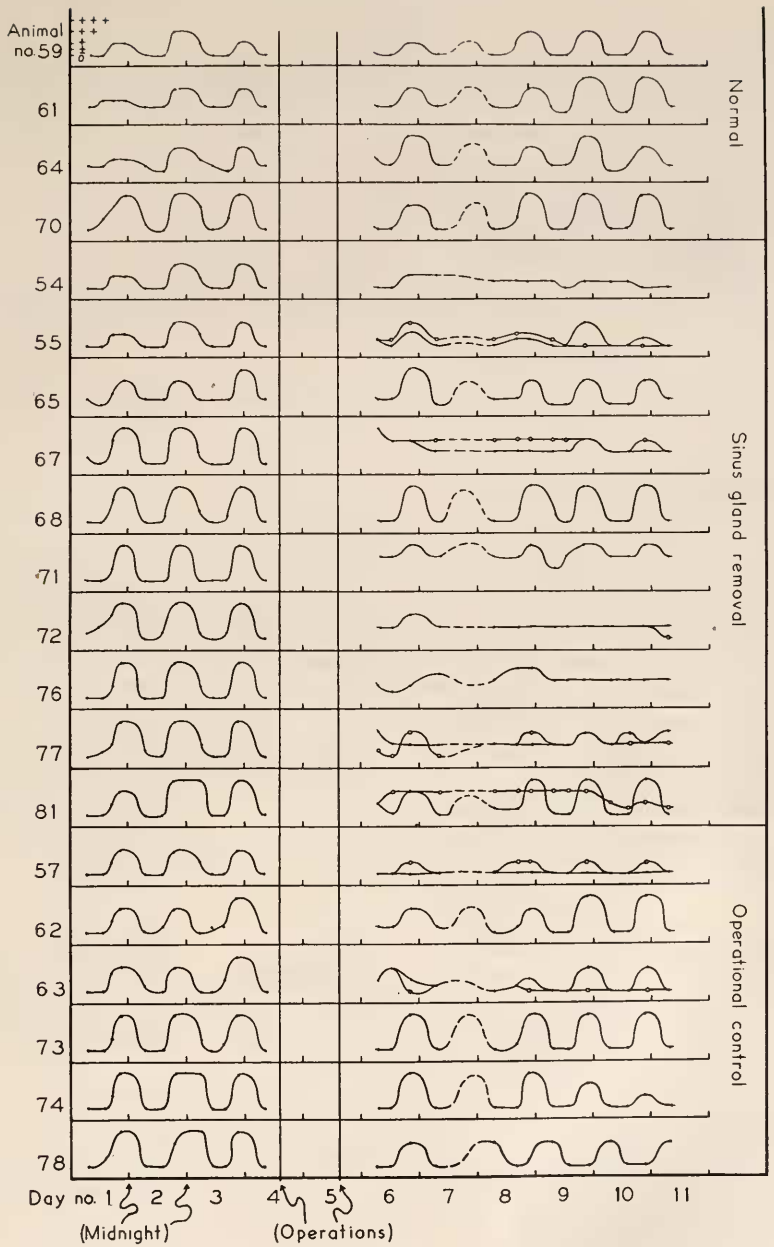

FIGURE 3. The effects of sinus gland removal and optic nerve damage upon the diurnal rhythm of retinal pigment migration of crabs kept in darkness. Double curves indicate a difference in the two eyes of one animal, the open circles indicating the left eye. Single curves indicate that both eyes were the same.

Table VI

Results of histological examination; sinus gland removal and control operations; continuous darkness

Record number	Species	Response	Sinus glands	Nerve damage
59	*H. nudus*	Normal (control)	—	—
61	*P. crassipes*	Normal (control)	—	—
64	*P. crassipes*	Normal (control)	—	—
70	*H. oregonensis*	Normal (control)	—	—
54	*H. nudus*	Right: approaching "day" state Left eye removed	Removed —	Optic tract severed —
55	*H. nudus*	Right: impaired "night" migrations Left: "day" condition	Right: trace Left: removed	Slight Severe
65	*P. crassipes*	Right: normal Left eye removed	Right: trace —	Slight —
67	*H. oregonensis*	Both: intermediate	Both: removed	Both: moderate
68	*H. oregonensis*	Both: normal	Right: trace Left: removed	Both: slight
71	*H. oregonensis*	Both: migrations, tending to "night" state	Both: removed	Both: moderate
72	*H. oregonensis*	Both: intermediate	Both: removed	Both: moderate
76	*H. oregonensis*	Right: intermediate Left eye removed	(?) —	(Lost in sectioning; no evaluation)
77	*H. oregonensis*	Both: intermediate, slight migrations	Both: removed	Right: severe Left: moderate
81	*H. oregonensis*	Right: normal Left: intermediate	Right: (?) Left: removed	Right: (lost in sectioning) Left: moderate
57	*H. nudus*	Right: "day" state Left: impaired night migrations	Both: intact	Right: optic tract severed Left: moderate
62	*P. crassipes*	Both: normal	—	(Not sectioned)
63	*P. crassipes*	Right: nearly normal Left: "day" condition	Both: intact	Right: slight Left: optic tract severed
73	*H. oregonensis*	Both: normal	Both: intact	Both: slight
74	*H. oregonensis*	Right: normal Left: (not operated upon)	Right: intact Left: (intact)	Right: slight Left: —
78	*H. oregonensis*	Both: normal	—	(Not sectioned)

eye only). The results were quite variable, nos. 54, 67, 72, and 76 exhibiting a loss of diurnal pigment movements; nos. 65, 68, and 71 continuing to show these changes; and nos. 55, 77, and 81 exhibiting diurnal rhythm in the pigments of one eye and not the other. Histological examination of the eyes in this group showed that there was great variation in the extensiveness of nerve damage in different cases. The following summary (Table VI) indicates the extent to which histological results (admittedly unsatisfactory) agree with the observed behavior.

The results shown in Figure 3 and Table VI bear out the earlier indications that severe damage to nerves can cause a persistent state of day-adaptation in the retinal pigments. The effects of sinus gland removal are less easy to evaluate. In the case of three animals (nos. 55, 65, and 68) the possibility of traces of the sinus gland remaining in the eyestalk cannot be eliminated. Even in cases where the gland itself is clearly absent, its nerve may be seen extending far into the medulla terminalis. Since the sinus gland nerve in life exhibits the same characteristic bluish appearance as the sinus gland itself, and shows in fixed material the presence of an eosinophilic secretion product, it must be admitted that it may well be capable of compensating to some extent for the loss of the gland proper. Perhaps of more significance are those animals (nos. 67, 71, 72, 76, 77, and the left eye of 81) in which sinus gland removal is followed by incomplete day-adaptation, and in which nerve damage, though present, is not excessive. Although sinus gland removal has failed, as before, to produce a full and persistent night-adapted state, it seems at least in these cases to have produced a shift in that direction. Since nerve damage tends to produce the day-adapted state, the intermediate condition may be the resultant of the effect of sinus gland removal promoting dark-adaptation and nerve damage causing light-adaptation. The fact that this "tendency" toward dark-adaptation is not wholly effective even in continuous darkness, does not in itself encourage one to assign an exclusive role in retinal pigment control to the sinus gland. Likewise the fact that the eyes of the same animal may behave differently is more easily attributable to unequal nerve damage than to sinus gland removal.

Discussion

The studies reported above were undertaken in an attempt to verify the generally accepted claim that day-adaptation in the decapod crustacean eye occurs under the influence of a hormone produced and released by the sinus glands. The work upon which this view is based has been done chiefly upon macrurans: shrimps and crayfish. The critical test of sinus gland removal still remains to be applied to the macrurans. Its application in the brachyurans, reported herein, while generally supporting previous contentions regarding the macrurans, does indicate that at least in the grapsoid crabs the sinus gland is not the sole source of retinal pigment activator. Despite numerous operations, simple sinus gland removal has usually failed either to cause the eye to remain dark-adapted or to cease its diurnal pigmentary changes, except that in continuous darkness there may be a lessened ability to assume the day-adapted condition during daylight hours. In all types of operations there has been shown a marked tendency for nerve damage to result in a more pronounced state of day-adaptation, a factor which should be taken into account when describing the effects of operations upon the sinus gland. The injection of extracts of other tissues indicates that while the sinus gland is clearly richer in active mate-

rial, nervous tissues contain large amounts, the sinus gland nerve showing visible secretion products like the sinus gland. If this material is utilized as a hormone, we are forced to consider the possibility that it can be released by nervous tissues in significant amounts, even in the virtual or complete absence of sinus glands.

Summary

1. The grapsoid crabs, *Hemigrapsus* and *Pachygrapsus,* exhibit in constant darkness a marked diurnal rhythm of retinal pigment migration.

2. This rhythm is absent in continuous light, and can be induced to become out of phase with solar day in constant darkness.

3. About ⅓ of the retinal pigment activator of the eyestalk resides in the sinus gland, with the remaining ⅔ distributed in the optic ganglia. Brain and other nervous tissues also contain an active principle in concentrations comparable to that in optic ganglia.

4. The sinus gland is at least 20 times richer than nervous tissue in retinal pigment activator.

5. Damage to nerves of the optic tract impairs the attainment of night-adaptation. If sufficiently severe, nerve damage may result in a state of permanent day-adaptation.

6. Operative sinus gland removal does not produce full dark-adaptation, a fact which may be in part explainable on the basis of concomitant nerve damage, but it does reduce the extent of day-adaptation in crabs kept in constant darkness.

7. It is concluded that, in the grapsoid crabs studied, the sinus glands are specialized (in addition to entirely different functions) for the elaboration and possibly the release of a principle active upon retinal pigments. On the present evidence it cannot be concluded that the sinus glands are the sole source of the retinal pigment activating hormone(s) in this group of crabs.

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